

## PROGRAMMABLE CONDITIONAL SIRNAS AND USES THEREOF

### PRIORITY CLAIM

**[0001]** This application is a continuation of International Patent Application No. PCT/US2019/046075, filed Aug. 10, 2019, which claims priority to U.S. Provisional Application No. 62/717,686, filed on Aug. 10, 2018, and 62/811,183, filed on Feb. 27, 2019, the contents of which are incorporated by reference herein in their entireties, including drawings.

### STATEMENT OF GOVERNMENT INTEREST

**[0002]** This invention was made with government support under grant number A1029329, awarded by the National Institutes of Health and grant numbers 1332411 and 1120890, awarded by the National Science Foundation. The government has certain rights in the invention.

### SEQUENCE LISTING

**[0003]** The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Feb. 10, 2021, is named Sequence-Listing.txt and is 22 kilobytes in size.

### BACKGROUND

**[0004]** A longstanding goal for nucleic acid nanotechnology<sup>1-3</sup> and biomolecular computing<sup>4</sup> is the development of conditionally activated oligonucleotide therapeutics that can detect and respond to cellular expression of specific genes<sup>3,4</sup>. Nucleic acid switches based on toehold mediated strand displacement<sup>5,6</sup> have executed logic operations and detected RNA transcripts in both bacterial and mammalian cells<sup>3,8</sup>, but the conditional activation of oligonucleotide drugs by RNA transcripts in mammalian cells has not been convincingly demonstrated. Significant challenges include poorly suppressed background drug activity, weak ON state drug potency, input and output sequence overlap, high design complexity, short device lifetimes (<24 hours) and high required device concentrations (>10 nM).

**[0005]** Over the past decade, synthetic RNAi triggers such as small interfering RNAs (siRNAs)<sup>10</sup> have become ubiquitous tools in biological research, and extensive basic and clinical development efforts have recently culminated in the FDA approval of ONPATRO, the first RNAi drug<sup>11</sup>. Despite a burgeoning drug development pipeline and an extensive compendium of excipients targeting ligands and delivery techniques<sup>9</sup>, the difficulty of delivering RNAi agents to specific populations of disease related cells continues to limit the potential of RNAi therapy. Repeated attempts over the past fifteen years to develop programmable, conditionally activated RNAi agents based on strand displacement switches<sup>12-15</sup> have not convincingly demonstrated the intended effects, despite notable progress<sup>3,8,16-19</sup>. Thus, a new conditionally activated siRNA (Cond-siRNA) is provided herein to overcome the problems in the art.

### SUMMARY

**[0006]** In one aspect, this disclosure relates to a programmable, conditionally activated small interfering RNA construct (Cond-siRNA) in an OFF state, the construct com-

prising a sensor strand, a core strand, and a guide strand, wherein the sensor strand and the core strand bind complementarily to form a sensor duplex, the guide strand and the core strand bind complementarily to form a RNAi duplex, and the sensor duplex and the RNAi duplex are attached to each other to form a single structure. In some embodiments, the sensor duplex and the RNAi duplex are attached to each other via the core strand. In some embodiments, the sensor strand complementarily binds to a fragment on the 5' and a fragment on the 3' of the core strand to form the sensor duplex, and the guide strand complementarily binds to a fragment in the middle of the core strand to form the RNAi duplex and the fragment in the middle does not comprise any 5' or 3' sequence of the core strand such that the sensor duplex and RNAi duplex are attached to each other via two different fragments of the core strand. In some embodiments, the sensor domain of the Cond-siRNA construct comprises a sensor duplex formed by complimentary binding of the sensor strand and 3' and 5' fragments of the core strand, and a sensor overhang that does not pair up with the core strand. The sensor overhang is either at the 3' end or at the 5' end of the sensor strand. In some embodiments, the sensor strand, core strand, and guide strand form the single construct via self-assembling upon contact of each other. In some embodiments, the sensor duplex comprises 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 base pairs. In some embodiments, the RNAi duplex comprises 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 base pairs.

**[0007]** In some embodiments, the Cond-siRNA is chemically modified to further improve the OFF-state stability and/or dissociation efficiency when turned on upon contact with an input strand. For example, the bases of the sensor strand in the duplex region is modified by LNA modification, 2'-O-methyl modification, or both but not by phosphorothioate (PS) modification; either or both termini of the core strand are modified with PS modification, 2'-O-methyl modification, or both; or the single strand overhang of the sensor strand is modified by LNA modification, 2'-O-methyl modification, PS modification, or any combination thereof. In some embodiments, the sensor domain and the RNAi domain are modified by different chemical modifications.

**[0008]** The OFF-state Cond-siRNA described above is activated or turned on by contacting the OFF-state Cond-siRNA with an input strand, wherein one end of the input strand forms a toehold with the sensor strand overhang to induce displacement of the sensor strand from the core strand via complementary binding of the input strand and the sensor strand to form a waste duplex, whereby the RNAi duplex is completely disassociated from the construct.

### BRIEF DESCRIPTION OF THE DRAWINGS

**[0009]** This application contains at least one drawing executed in color. Copies of this application with color drawing(s) will be provided by the Office upon request and payment of the necessary fees.

**[0010]** FIGS. 1a-d show conceptual design, operation, and molecular dynamics simulation of Cond-siRNAs. FIG. 1a shows the conceptual secondary and tertiary structure of the Cond-siRNA. Docking of the RNAi duplex to an x-ray crystal structure of Giardia Dicer shows massive steric clashes between the sensor duplex and Dicer. FIG. 1b shows RNAi activation via strand displacement. When a complementary input RNA meets the Cond-siRNA (I), the input